Chromosomal and genetic abnormalities in myeloma

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Summary Chromosomal translocations are a hallmark of lymphoid tumours. Multiple myeloma (MM) is a tumour of the plasma cell, the terminally differentiated B lymphoid cell. In recent years, a large number of chromosomal and genetic abnormalities have been detected in myeloma, the most prominent being chromosome 13q deletions and translocations affecting the immunoglobulin heavy chain (IgH) locus on chromosome 14q32. The latter involve a large array of chromosomal partners, from which multiple oncogenes have been proposed as candidates for dysregulation. In addition, a wide variety of changes including numerical aberrations, translocations involving loci other than the immunoglobulin genes, and aberrations of known oncogenes such as N-ras mutations, have been found. With the refinement of molecular cytogenetic techniques, the sensitivity of detecting these molecular abnormalities is continuing to increase. However, with the exception of 13q deletions which have been consistently associated with an adverse prognosis, the role of the other changes in the pathogenesis of MM, and their effect on disease behaviour and prognosis are still being clarified. In this review, we will discuss the most common molecular abnormalities found in primary MM and cell lines, and consider the available evidence for a pathogenic role in MM.

Keywords Myeloma, MGUS, chromosomal translocation, karyotype, oncogenesis

Introduction

Multiple myeloma (MM) is a malignancy of the plasma cell, the terminally differentiated B cell. Early investigations of the chromosomal abnormalities of MM were based on conventional karyotype analysis, with the detection of several recurrent abnormalities such as monosomy 13 and the t(11; 14) translocation, the nature of which has been elucidated by molecular techniques. In more recent years, developments in molecular cytogenetics ranging from metaphase and interphase fluorescent in situ hybridization (FISH) to multicolor spectral karyotyping (SKY) and comparative genomic hybridization (CGH) have vastly increased the scope of detection of cytogenetic abnormalities. The clinical and prognostic implications of these changes are still to be fully elucidated, while specific genetic aberrations have increased our understanding of the pathogenesis of this malignancy.

I. Monosomy 13

Monosomy 13 was one of the first recurrent chromosomal abnormalities found in MM, detectable in many cases by conventional karyotype, and for which adverse prognostic significance has been consistently established (Tricot et al., 1995; Perez-Castro et al., 1997; Zojer et al., 2000). This has been confirmed by interphase FISH studies, in which monosomy 13 was found to be the most powerful predictor of survival (Königsberg et al., 2000; Facon et al., 2001), especially in combination with β2 microglobulin (Facon et al., 2001). Because of the adverse prognostic impact of monosomy 13 shown in these studies, it has been postulated to be a late determinant of disease progression rather than an oncogenic factor. However, with the development of molecular cytogenetics, the frequency of detection of monosomy 13 has also risen...
from approximately 15–20% by conventional cytogenetics (Lai et al., 1995; Seong et al., 1998; Desikan et al., 2000) to approximately 50% by FISH (Avet-Louseau et al., 2000; Fonseca, Oken & Greipp, 2001) and SKY (Sawyer et al., 2001), with an incidence of 30–45% also found in monoclonal gammopathy of unknown significance (MGUS) (Königsberg et al., 2000; Fonseca et al., 2001). Moreover, in the molecular cytogenetic studies, monosomy 13 was often found in conjunction with a large variety of other chromosomal aberrations, and the prognostic significance of monosomy 13 alone was more difficult to define. For example, it was observed that while all patients carrying translocations involving the IgH locus such as t(4;14) and t(14;16) were found to have del 13, the reverse was not true (Sawyer et al., 1998; Fonseca et al., 2001). This suggests the possibility that del 13 occurred before the IgH translocation event, possibly as an early oncogenic change. A role for monosomy 13 in the transformation of MGUS to MM has also been proposed, supported by a higher incidence of monosomy 13 in MM cases with pre-existing MGUS (70%) compared with those cases without such a history (31%) (Avet-Loiseau et al., 1999a). In addition, while monosomy 13 occurs in the majority of plasma cells in a MM tumour, in MGUS it is only present in subpopulations (Avet-Loiseau et al., 1999b).

Although the majority (92%) of chromosome 13 abnormalities are monosomies, partial deletions are also recognized (Avet-Loiseau et al., 2000). In the latter, the region of deletion has been localized to 13q14–21, overlapping the minimal region of deletion in chronic lymphocytic leukaemia (Cigudosa et al., 1998; Avet-Loiseau et al., 2000). The retinoblastoma gene (Rb) is located on chromosome 13q and its role has been examined in MM. Despite the frequency of monosomy 13 and monoallelic Rb loss (up to 60% of tumours and cell lines), abnormalities in Rb expression are rare and not correlated with monosomy 13. Rb gene rearrangements or mutations (Zandecki et al., 1995). It is therefore unlikely that monosomy 13 acts solely through Rb.

II. Immunoglobulin heavy chain (IgH) switch translocations

A. Isotype switch recombination

During B cell maturation, the IgH genes undergo the processes of variable region (VDJ) recombination, followed by isotype switching and somatic hypermutation after exposure to antigen. Isotype switching is the process by which a given Ig variable region can be associated with different isotype classes which confer different physiological functions. Its particular relevance in MM lies in the fact that the majority of translocations affecting the IgH locus on chromosome 14q32 occur at the switch regions where normal isotype switch recombinations occur, and are known as switch translocations (STs) (see Figure 1) (Bergsagel et al., 1996). The IgH genes are arranged in the order of 5′–Cμ, Cδ, Cγ3, Cγ1, Cψε, Cα1, Cγ2, Cγ4, Cε, Cα 2–3′. Switch regions, consisting of repeat sequences, are located in front of each constant region gene except Cδ. The first heavy chain isotype IgM is expressed by RNA splicing from the end of the rearranged variable region to the Cμ gene. During isotype switch recombination the μ isotype changes to another isotype by recombination of the respective switch regions. For instance, Sμ recombines with Sα for the μ to α switch, with the deletion of the intervening DNA in an excision circle. Switch recombination mostly involves switch regions on the same chromosome (in cis), while trans-switching involving switch regions on both alleles may also occur more rarely. The fact that switch regions are physiological recombination hotspots may account for their propensity to be involved in chromosomal translocations. The retention of Ig production by the myeloma plasma cell would imply that the STs are most likely to occur on the nonproductive allele.

B. Switch translocations in myeloma

Apart from monosomy 13, the most common cytogenetic abnormality in MM is chromosomal translocation into the switch regions of the IgH genes on chromosome 14q32. These translocations were initially found by conventional cytogenetics at a rate of 20–40% (Taniwaki et al., 1994; Lai et al., 1995; Taniwaki et al., 1996). The partner chromosome was often not elicited, and the karyotypic abnormality was denoted 14q+. Using a southern blot technique to look for illegitimate switch recombinations (ISRs) as candidates for switch translocations (STs) in human myeloma cell lines (HMCLs), it became evident that STs were much more common than previously suspected (Bergsagel et al., 1996). It is estimated that approximately 90% of HMCLs have either IgH or IgL (immunoglobulin light chain) translocations. Cloning of breakpoints and molecular cytogenetic studies have revealed a large array of chromosomal partners for the STs, the most common being chromosomes 11q, 4p, 16q and 6p, with candidate oncogenes characterized on each partner. The translocation breakpoints are mostly centromeric to the candidate oncogenes, which are translocated to der(14) and placed under the control of one of the
IgH enhancers (Eرأر or Eرأض). In addition, in cases such as chromosome 4p, a second putative oncogene is also overexpressed on der(4), presumably dysregulated by the 5¢ intronic enhancer (Eرأع) translocated to der(4). The role of these candidate genes in the pathogenesis of myeloma has been examined by assessing their level of expression and their oncogenicity in both in vitro and in vivo models.

C. Translocation partner chromosomes

The most common switch translocation partners and the associated candidate oncogenes are listed in Table 1.

1. Chromosome 11q

Chromosome 11q has been identified as a partner chromosome in 15–20% of MM (Avet-Loiseau et al., 1998; Fonseca et al., 1998), with translocations located at 11q13. Early studies suggested that this translocation was associated with a poor prognosis (Tricot et al., 1995; Fonseca et al., 1998). However, these results were obtained by conventional cytogenetics which require metaphases from actively dividing cells, possibly 'selecting' for more aggressive disease. Subsequent studies by interphase FISH have not confirmed the prognostic relationship (Avet-Loiseau et al., 1998).

Chromosome 11 ST breakpoints are not as telomeric compared with other partner chromosomes such as chromosome 4p, and are therefore more amenable to detection by conventional cytogenetics. Although mantle cell lymphoma (MCL) also bears a characteristic t(11; 14)(q13;q32) translocation, the majority of MCL breakpoints are located within the major translocation cluster (MTC) region 110 kb upstream of Cyclin D1 (CD1, candidate oncogene for both MCL and MM). In contrast, the breakpoints in MM are more widely scattered over a region 100–330 kb centromeric to CD1. In MM, except for several breakpoints in the JH region, most of the known IgH breakpoints occur in the switch regions. In contrast, in MCL they are all located in the JH region, indicating the involvement of the VDJ recombination mechanism at an earlier stage of B cell development.
The finding of CD1 up-regulation in MM tumours and cell lines bearing t(11; 14) supports its role as a candidate oncogene in MM (Chesi et al., 1996; Hoyer et al., 2000; Pruneri et al., 2000). The Cyclins are a family of cell cycle regulators, of which the members CD2 and CD3 are normally expressed in lymphoid cells. The expression of CD1 is therefore ectopic in t(11; 14) bearing MM cells. All the Cyclin D proteins interact with CD-dependent kinases, phosphorylating and inactivating the retinoblastoma protein (Rb), thus promoting the G1/S phase transition (Sherr, 2000).

A second candidate oncogene named myeov has been characterized on chromosome 11q, 360 kb centromeric to Cyclin D1, with all the known MM breakpoints situated in the region between CD1 and myeov (Janssen et al., 2000). Myeov was originally isolated from gastric carcinoma by a tumorigenicity assay. In MM, myeov was up-regulated in 3 of 7 cell lines carrying t(11; 14), indicating that not all myeloma lines carrying the translocation overexpressed myeov. The variation is presumably related to the site of the breakpoint, the orientation of the translocation and the placement of enhancers. Surprisingly, some cell lines not carrying t(11; 14) express moderate levels of myeov (Janssen et al., 2000). Hence the role of myeov in MM is still not clear.

2. Chromosome 4p
The t(4; 14)(p16;q32) translocation is difficult to detect by conventional cytogenetics or SKY (Rao et al., 1998; Sawyer et al., 1998; Sawyer et al., 2001), due to the extreme telomeric localization of the breakpoints. The cloned chromosome 4 breakpoints were located 50–100 kb centromeric of FGFR3, one of the 2 candidate oncogenes (Chesi et al., 1997; Richelda et al., 1997). The translocation can be detected by FISH, with the reported incidence varying from 12% (Avet-Loiseau et al., 1998) to 17% (Finelli et al., 1999) in MM. It is also detected in 2–6% in MGUS (Avet-Loiseau et al., 1999a; Malgeri et al., 2000), the latter estimate was obtained by the demonstration of a fusion RNA transcript derived from chromosomes 4 and 14 (see below).

FGFR3 is one of a family of fibroblast growth factor receptor tyrosine kinases, mutations of which cause several forms of dwarfism including thanatropic dysplasia (TD) (Naski et al., 1996; Webster et al., 1996). FGFR3 can be dysregulated in MM by translocation to the vicinity of IgH enhancers, activating mutations, or both. It is overexpressed in several MM cell lines and tumours, with selective expression of the mutant allele in the cell lines carrying FGFR3 mutations (Chesi et al., 2001). The frequency and role of FGFR3 mutations in primary MM tumour is much debated, due to reports of a low incidence in primary tumour (Fracchiolla et al., 1998; Intini et al., 2001). In FGFR3 genomic DNA, a single nucleotide change was found in one of 80 primary MM tumours (Fracchiolla et al., 1998). A low incidence was also detected in cDNA from the expressed allele – only one of 11 cases overexpressing FGFR3 demonstrated an activating mutation (Intini et al., 2001). It was concluded from these studies that the incidence of activating mutations of FGFR3 in MM was 10% in cases bearing t(4; 14), which constitutes only 2% of all MM primary tumours.

Possible mechanisms of action of FGFR3 in MM have been extensively investigated. In TD, FGFR3 mutations were shown to cause constitutive phosphorylation of the receptor, leading to ligand-independent activation (Webster et al., 1996). By retroviral transduction of wild-type

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Table 1. Common chromosomal partners and candidate oncogenes in switch translocations in myeloma

<table>
<thead>
<tr>
<th>Chromosomal partner</th>
<th>Candidate Oncogene</th>
<th>Localization</th>
<th>Distance between breakpoints &amp; telomeric oncogenes</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>11q13</td>
<td>1. Cyclin D1</td>
<td>der(14)</td>
<td>100–330 kb</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td></td>
<td>2. myeov</td>
<td>der(11)</td>
<td></td>
<td>unknown</td>
</tr>
<tr>
<td>4p16</td>
<td>1. FGFR3</td>
<td>der(14)</td>
<td>50–100 kb</td>
<td>Growth factor receptor tyrosine kinase</td>
</tr>
<tr>
<td></td>
<td>2. MMSET/WHSC1</td>
<td>der(4)</td>
<td></td>
<td>Epigenetic regulator of transcription (chromatin remodelling)</td>
</tr>
<tr>
<td>16q32</td>
<td>c-maf</td>
<td>der(14)</td>
<td>550–1350 kb</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>6p21</td>
<td>Cyclin D3</td>
<td>der(14)</td>
<td>65 kb</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>6p25</td>
<td>MUM1/IRF4</td>
<td>der(14)</td>
<td>Immediately adjacent</td>
<td>Transcriptional regulator of IFN &amp; IFN-stimulated genes</td>
</tr>
</tbody>
</table>

IFN: interferon.
and mutant FGFR3 into murine IL6-dependent B9 plasmacytoma cell line, increased levels of FGFR3 expression (both wild-type and mutant) increased cellular proliferation and survival, with an enhanced response to IL6 and IL6-independence (Plowright et al., 2000). Mutant FGFR3-transduced cells also exhibited independence of ligand (FGF). However, the increased cellular proliferation and survival from up-regulated expression of wild-type FGFR3 would appear to be dependent on the level of FGFR3 expression and not specifically on the mutation. IL6 has both mitogenic and anti-apoptotic effects on MM cells, the former is promoted primarily by the mitogen-activated protein kinase (MAPK) pathway, and the latter by the JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathway with up-regulation of bcl-xL through gp130 as an intermediary (Schwarze & Hawley, 1995). Plowright et al. (2000) found that in the absence of gp130 and IL6, up-regulated FGFR3 increased phosphorylation of STAT3, which then up-regulated bcl-xL to reduce apoptosis. There was no evidence of MAPK involvement as an explanation for FGFR3-induced IL6-responsive cellular proliferation.

In contrast, FGFR3 dysregulation was found to affect the MAPK pathway in other experiments. Ronchetti et al. (2001) examined three cell lines containing ‘native’ mutant FGFR3 and demonstrated phosphorylation of MAPK in all 3 lines, even though only two of them showed constitutive activation of FGFR3. Neither STAT1 or STAT3 were involved. Chesi et al. (2001) showed that the stimulation of three t(4; 14)-bearing cell lines by FGF also resulted in rapid MAPK phosphorylation. The oncogenicity of mutant FGFR3 was demonstrated by an in vitro focus formation assay and an in vivo animal model using a mutant FGFR3-transfected NIH 3T3 fibroblast cell line. As the MAPK pathway is known to be mediated by ras (Kanai et al., 1997), a dominant negative form of ras was used to inhibit the FGFR3 oncogenic effect, demonstrating involvement of both MAPK and ras as previously shown. Mutant FGFR3 has been found to cause transformation in haemopoietic cells, but whether up-regulated levels of nonmutated FGFR3 may have the same effect is presently unclear (Li et al., 2001). FGFR3 and ras mutations appeared to be mutually exclusive in MM, and may therefore have overlapping roles in myeloma pathogenesis (Chesi et al., 2001). However, as mentioned earlier, the low incidence of FGFR3 mutations in primary MM must be considered (Fracchiolla et al., 1998; Intini et al., 2001).

A second candidate gene for t(4;14) was localized on the reciprocal chromosome [der(4)]. The chromosome 4p breakpoints are telomeric to or within the 5’ introns of MMSET/WHSC1, characterized as a candidate gene for a multiple malformation syndrome known as Wolf-Hirschhorn Syndrome (Stec et al., 1998). MMSET is a member of the trithorax nuclear proteins which includes MLL, one of the most common genes involved in human acute leukaemia. MLL is involved in the epigenetic regulation of gene expression through chromatin remodelling, and consists of PHD (zinc finger) and SET domains. The latter has been shown to cause transformation in fibroblasts (Cui et al., 1998). In t(4;14) MMSET is placed in close proximity to and presumably up-regulated by the 5’ intron IgH enhancer. Hybrid transcripts of 5’IgH joined to MMSET are produced, initiating either from the JH1 or JH exons (Chesi et al., 1998b; Malgeri et al., 2000). However few of the fusion transcripts are expected to produce functional proteins due to premature stop codons, or the absence of the amino terminal as a result of splicing. The size of proteins could also vary according to the use of different promoters, alternative splicing, specific mRNA degradation and variation in the site of polyadenylation. Although it has been proposed that such variations may modulate the phenotypic diversity of MM (Stec et al., 1998), so far there has been no evidence for a correlation with clinicopathological characteristics.

3. Chromosome 16q
The t(14;16)(q23;q23) translocation has been found in approximately 5–12% of MM (Sawyer et al., 1998; Sawyer et al., 2001). The chromosome 16q23 breakpoints were localized to a region 550–1350 kb centromeric of the candidate oncogene c-maf, although a breakpoint telomeric to c-maf was also found in a t(16; 22) translocation (Chesi et al., 1998a; Bergsagel & Kuehl, 2001). c-maf is a basic zipper transcription factor involved in cellular differentiation, proliferation and the IL-6 response, and is capable of oncogenic transformation in a model system (Kataoka et al., 2001). Its role in myelomagenesis is supported by the finding of upregulated expression in five HMCLs carrying the t(14;16) translocation including the tumour of origin of one cell line, with selective expression of one c-maf allele in two informative lines (Chesi et al., 1998a). The cloned breakpoints are located in a common fragile site (FRA16D) on chromosome 16, within the newly characterized WWOX gene, a candidate tumour suppressor gene in breast cancer (Bednarek et al., 2000). Although this region is susceptible to allelic deletion in several non-haematological cancers, no mutations have been found in the remaining allele of WWOX, and its possible role as a tumour suppressor gene is not yet known. Similarly its role in MM is unclear, as breakpoints occurring within WWOX would inactivate only one of the two alleles.
4. Chromosome 6p
The t(6;14)(p25;q32) translocation was one of the first STs reported in a HMCL (Iida et al., 1997), at which MUM1/IRF4, an interferon (IFN)-responsive factor (IRF), was proposed as a candidate oncogene. IRFs are involved in transcriptional control, can be rapidly induced by T and B receptor cross linking, and regulate the IFNs and IFN-stimulated genes. They therefore have a critical role in plasma cell development (Mittrucker et al., 1997). IRF4 was overexpressed in HMCLs and demonstrated transforming ability in fibroblasts (Iida et al., 1997). Molecular cytogenetics have revealed an incidence of this ST of 18% in HMCLs (Yoshida et al., 1999) but a low frequency of approximately 5% in primary MM (Sawyer et al., 1998; Sawyer et al., 2001), and its role in MM pathogenesis is as yet unclear.

More recently, a second translocation affecting chromosome 6p–t(6;14)(p21;q32) was identified. Cyclin D3 (CD3), located approximately 65 kb telomeric of the breakpoint at 6p21, has been proposed as the candidate oncogene (Shaughnessy et al., 2001). This translocation was originally found in one of 30 myeloma cell lines, and then in 4% of 150 primary tumours by metaphase FISH and SKY. CD3 overexpression was further detected by microarray analysis in 3 of 53 primary tumours, in which t(6;14) breakpoints were subsequently confirmed, one other tumour having a variant t(6;22) translocation. As noted earlier, all the Cyclin D proteins are known to phosphorylate Rb, facilitating the G1/S phase transition. Unlike CD1, CD3 is normally expressed in lymphoid cells and upregulated expression would be expected to promote cellular proliferation.

5. Other chromosomal partners
The c-myc on chr 8q24 is the oncogene characteristically dysregulated in murine plasmacytoma and human Burkitt’s lymphoma. Reciprocal translocations involving chromosome 8q24 and the Ig genes account for only 25% of the c-myc rearrangements in MM (Avet-Loiseau et al., 2001b), with the majority involving non-Ig loci. Hence c-myc rearrangements will be discussed below with the non-Ig translocations (see Section IIIb).

Apart from the more common STs already discussed, a large number of other chromosomal partners have been reported, many detected only once. Reciprocal translocations with chromosome 1q have been detected by SKY, accounting for up to 4–5% of primary tumours (Sawyer et al., 1998). Possible candidate oncogenes on chromosome 1q are IRTA1 and 2, two novel B-cell surface receptors. IRTA2 has been found to be upregulated in t(1;14)-carrying tumour cell lines (Hatzivassiliou et al., 2001). There are many other infrequent partners including 3q, 4q, 20q, 21q and 22q. Due to the nonrecurrrent nature of some of these aberrations, their role in MM pathogenesis would be difficult to define.

D. Switch translocations – implications for pathogenesis?
Whether STs are an early oncogenic change, a late ‘trigger’ of disease progression or a non-pathogenic marker of genetic instability has been an interesting question with important implications on our understanding of myeloma pathogenesis. If STs are oncogenic, their presence in 50–75% of primary MM tumours (Nishida et al., 1997; Avet-Loiseau et al., 1998; Sawyer et al., 1998; Ho et al., 2001) and the heterogeneity of chromosomal partners and partner oncogenes would imply that STs cannot be the only oncogenic factor, and a number of mechanisms must be involved. This heterogeneity was proposed as one of the arguments against STs being a unifying oncogenic change (Avet-Loiseau et al., 1998). However, the ‘final common pathway’ of myeloma development from multiple mechanisms could simply reflect a single possible phenotype of the transformed plasma cell (Bergsagel & Kuehl, 2001).

The concept of primary and secondary translocations has been introduced to distinguish translocations that occur early in disease and may be oncogenic, from late changes which may play a role in disease progression (Bergsagel & Kuehl, 2001). Translocations such as STs which involve B cell recombination mechanisms, occur in some cases of MGUS, and demonstrate little heterogeneity within the myeloma cell population of each tumour, are more likely to represent primary changes. Conversely, changes which do not involve the Ig loci, are not present in MGUS and demonstrate heterogeneity within a tumour are more likely to represent late, secondary changes. These may include the complex c-myc translocations, which do not occur at the Ig recombination sites and demonstrate intratumour heterogeneity (see Section IIIb).

An interesting consideration in the role of STs in the causation of MM is their occurrence in MGUS. Not all MGUS are premalignant, and there are presently no clearly defined molecular or other phenotypic features which enable us to predict the risk of MM development. If STs are an oncogenic change, the presence of STs may distinguish the benign and premalignant forms of MGUS.

In the largest series of MGUS patients assessed by FISH so far (Avet-Loiseau et al., 1999a), chromosome 14q32 translocations have been found to occur in 47%, with a

progression to 60% in intramedullary MM (Stage III) and 70–80% in PCL (Avet-Loiseau et al., 2001a).

The study of when STs occur in the ontogeny of the myeloma plasma cell can help to elucidate the cell of origin of the myeloma clone. Some studies have suggested that MM may originate in a pre-switch B cell or pre-plasma cell. These observations include the finding of pre-switch clonotypic cells, clonotypic IgM and nonclinical clonotypic iso-
types (Corradini et al., 1993; Bakkus et al., 1994; Billadeau et al., 1996; Szczepak et al., 1998; Reiman et al., 2001). As STs are commonly thought to occur during isotype switch recombination, the finding of pre-switch clonotypic cells raises the interesting question of whether these presumed myeloma precursors contain STs or not.

III. Other chromosomal abnormalities in myeloma

It is evident in molecular cytogenetic studies of MM that there are multiple structural abnormalities which do not affect the Ig loci (Cigudosa et al., 1998; Rao et al., 1998; Sawyer et al., 1998; Sawyer et al., 2001). The nonrecurrent nature of such changes makes analysis of their role in the disease difficult, and it is possible that a substantial number may represent ‘by-products’ of genetic instability.

A. Numerical changes

Numerical changes (monosomy, trisomy) have been a prominent finding in MM in conventional and molecular cytogenetic studies, detected in up to 80–90% cases (Drach et al., 1995; Cigudosa et al., 1998). The most common abnormality, monosomy 13, has already been discussed (Section I). Other recurrent aberrations include gains of chromosome 3, 5, 7, 9, 11 and 19. Comparative genomic hybridization (CGH) is a relatively new technique, by which tumour DNA is hybridized to normal metaphases and copy number changes are determined by comparison with reference DNA (Cigudosa et al., 1998). One CGH study showed numerical changes in 70% of cases, the most common being a gain of chromosome 19, with deletions of chromosome 13 in 30%. Multiple other changes were detected including del 6q (13%) and 16q (17%), some of which could carry tumour suppressor genes. Two changes previously associated with poor prognosis – gain of 11q and deletion of 13q – were detectable by CGH (Cigudosa et al., 1998).

B. Chromosome 8q and c-myc rearrangements

As noted earlier, the majority of translocations affecting c-myc in MM involve non-Ig loci. Estimates of the incidence of c-myc dysregulation varies. Shou et al., (2000) examined 20 myeloma cell lines by FISH for c-myc rearrangements (involving both Ig and non-Ig loci) and found a high incidence of 95%. Many were highly complex, with nonreciprocal translocations, multiple deletions and duplications. Dysregulated mono-allelic expression was demonstrated in all informative cell lines (when the 2 alleles could be distinguished). In contrast, Avet-Loiseau et al. (2001b) found a lower incidence (55%) of c-myc rearrangements in cell lines by FISH, and suggested that some of the previously described changes could be the result of in vitro propagation. In primary MM tumour, the reported incidences of c-myc rearrangements also varies from 15% in a large series of MM and primary PCL (Avet-Loiseau et al., 2001b), to 50% in advanced MM (Shou et al., 2000). In the former series, the rearrangements appeared to occur independently of disease stage, and there was no significant difference in incidence between patients at diagnosis (16%) and relapse (10%). Rearrangements involving Ig loci accounted for 25%. Due to the complexity of the chromosomal abnormalities and the involvement of non-B-cell specific mechanisms, c-myc rearrangements have been considered to represent late changes of disease progression. If this were the case, then c-myc rearrangements should correlate with disease stage. Although no such relationship was evident in the analysis of active MM cases so far, a correlation was found between c-myc dysregulation and β2 microglobulin, a poor prognostic indicator (Avet-Loiseau et al., 2001b). There was also intratumour heterogeneity in the occurrence of c-myc rearrangements, detected in 19% to 100% of plasma cells, supporting their role as secondary translocations. The incidence of c-myc rearrangements in MGUS (3%) and smouldering MM (4%) were also much lower than active MM (Avet-Loiseau et al., 2001b).

C. Other molecular aberrations not affecting the Immunoglobulin loci

In a recent SKY study, a large number of rearrangements not involving the Ig loci were detected, such as chromosomes 11q and 8, as well as complex translocations involving 3 or more chromosomes and whole-arm translocations (Sawyer et al., 2001). More than 50% of patients with complex karyotypes have coexistent chromosome 13 aberrations. Structural changes of chromosome 1q also feature prominently (Sawyer et al. 1995; Weh et al., 1995), even though reciprocal translocations involving the IgH locus and chromosome 1 are relatively scarce.
Mutations of N-ras and K-ras have been reported in approximately 40% of MM patients (Neri et al., 1989; Billadeau et al., 1995). They occur rarely in MGUS, and are associated with disease progression and resistance to therapy (Neri et al., 1989; Billadeau et al., 1995). Ras mutations are therefore likely to represent late, secondary changes in MM pathogenesis. In vitro studies have demonstrated IL-6 independence of HMCLs bearing ras mutations (Billadeau et al., 1995). Aberrations of p53, the tumour suppressor gene on chromosome 17p13, have also been postulated to play a role in MM pathogenesis. Although there are reports of p53 deletions as a predictor of poor survival, and del 17p13 has also been associated with poor prognosis (Drach et al., 1998), most studies indicate a low incidence of both p53 mutations and deletions in MM, varying from 3 to 9% (Preudhomme et al., 1992). The higher frequency of p53 mutations in PCL (20–40%) may indicate their role as a late molecular aberration in MM progression (Neri et al., 1993).

IV. Prognostic significance of switch translocations and other molecular abnormalities – an overview

By conventional cytogenetics, the most consistent chromosomal abnormality associated with poor prognosis is del 13, including both complete and partial deletions (Tricot et al., 1995; Perez-Castro et al., 1997; Zojer et al., 2000). This finding has since been duplicated in some molecular cytogenetics series (Königsberg et al., 2000; Facon et al., 2001). Conventional karyotyping has also demonstrated a poor prognostic significance of chromosome 11q translocations and reciprocal translocations with chromosomes 8, 9 and 12 (Tricot et al., 1995; Fonseca et al., 1998). However, this was not confirmed by molecular cytogenetics (Avet-Loiseau et al., 1998), and the possibility that conventional karyotypes have ‘selected’ out cases which are ‘hyperproliferative’ must be considered, especially when long-term cultures were used (Lai et al., 1995). The combination of aberrations of both chromosomes 11 and 13 has been found to produce a dismal outcome (Tricot et al., 1995).

In a study utilizing the detection of illegitimate switch recombinations (ISR) as an indication of STs in primary MM tumours, no relationship with parameters of disease activity or prognosis was found in patients with progressive disease (Ho et al., 2001). It was concluded that as a single entity, STs were unlikely to be a feature of disease progression or have prognostic significance, but subgroup analysis according to translocation partner is obviously required. This has been most efficiently examined by molecular cytogenetics. No correlation was found between the presence of chromosome 14q32 translocations and disease stage or β₂ microglobulin in a study of 127 MM and 14 PCL patients (Avet-Loiseau et al., 1998). In another analysis, Königsberg et al. (2000) divided 89 patients into three risk categories according to three chromosomal abnormalities – del 13q, abnormalities of chromosomes 11 and del 17q. When these factors were assessed separately, the only independent prognostic feature was del 13, with increased prognostic significance in conjunction with β₂ microglobulin. A recent study of PCL and late stage MM demonstrated a higher incidence of t(11;14), t(14;16) and monosomy 13 in patients with high tumour mass, but no difference in t(4;14). Paradoxically, a longer survival was observed in patients with t(11;14) in this group (Avet-Loiseau et al., 2001a). Further investigation is obviously still required to elucidate the prognostic significance of ST subgroups.

Conclusion

In conclusion, an overview of the molecular aberrations of MM is useful in illustrating the extremely complex abnormalities in this malignancy. The clear distinction of those changes which are oncogenic, from those which may promote tumour progression, and others which may be markers of genetic instability will be the subject of continued investigation, with important implications for our understanding of pathogenesis as well as management of the disease.

Acknowledgements

I acknowledge the encouragement and support of Professors Douglas Joshua and Antony Basten, and all members of the Myeloma Research Unit, Royal Prince Alfred Hospital. P.J.H. is supported by the University of Sydney Cancer Research Fund and the Royal College of Pathologists of Australia.

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